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ORIGINAL ARTICLE

Synthesis of 3-(pyridine-2-yl)-4,5-dihydro-1H-pyrazole-1-thiocarboxamides and their copper(II) complexes

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X-ray analysis;
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Abstract New organic ligands 3-(pyridine-2-yl)-4,5-dihydro-1H-pyrazole-1-thiocarboxamides (L) have been synthesized by a two or three step reaction sequence starting from substituted benzaldehydes and 2-acetyl pyridine. Copper(II) complexes with $LCuCl_2$ composition were obtained by the reactions of these ligands with $CuCl_2{\cdot}2H_2O$. The crystal structure of two synthesized complexes has been determined by X-ray analysis. Copper atoms located in a strongly distorted square piramidal environment and coordinated by pyrazoline and pyridine nitrogen atoms, thiocarbamoyl sulfur atom and two chloride-anions. All complexes undergo reversible or quasi-reversible electrochemical reduction at 0.45–0.28 V with the formation of Cu(I) containing intermediates. The cytotoxicity of copper containing complexes, comparable to cisplatin and doxorubicin, was demonstrated using cancer cell lines MCF-7, A549 and HEK293.

1. Introduction

2-Pyrazolines are an important class of heterocycles because of their high biological and pharmacological activities (Korablina et al., 2016). A recent approach in the treatment of many serious diseases such as cancer, AIDS, cardiovascular diseases and Alzheimer's disease is the development of drugs with multiple targets (Abdolmalekia and Ghasemi, 2016; Gökhan-Kelekçi et al., 2007; Slikker et al., 1999). Substituted pyrazolines and their derivatives are of interest because of their anti-bacterial, anti-fungal, and anti-inflammatory activities (Abdellatif et al., 2015; Abid et al., 2009; Abid and Azam, 2005a, 2005b; Budakoti et al., 2007, 2006; Fathalla et al., 2003; Palaska

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et al., 2001; Turan-Zitouni et al., 2005). It has been reported that 3,5-diaryl-4,5-dihydro-1-thiocarbamoyl-1H-pyrazole derivatives possess considerable anti-inflammatory activity with no ulcerogenic effects (Barsoum and Girgis, 2009). Additionally, many analogues have been found to be highly inhibitory against monoamine oxidases A (MAO-A) and B (MAO-B) isoforms, which suggest efficiency in treating Alzheimer's disease (Gökhan-Kelekçi et al., 2007) and use as anticonvulsants (Beyhan et al., 2017) and antidepressants (Özdemir et al., 2015).

N-Thiocarbamoyl-2-pyrazolines have been shown to exhibit antitubercular properties against *Mycobacterium tuberculosis* H37Rv (Ali et al., 2007), as well as antidepressant and anticonvulsant properties (Bilgin et al., 1993; Chinnaraja et al., 2016; Gökhan et al., 2003; Özdemir et al., 2007; Ruhoglu et al., 2005). Pyrazole derivatives containing 1-carbothioamide have exhibited EGFR inhibitory activity comparable to that of erlotinib. Furthermore, antiproliferative assay results have shown that some of these pyrazole derivatives have high antiproliferative activity against MCF-7 (Lv et al., 2010) and A549 (Yang et al., 2013) cell lines. Some N-Thiocarbamoyl-2-pyrazolines have also been shown to possess an antiepileptic activity (Vinayaditya et al., 2011).

In some cases, metal complexes of organic ligands have shown greater biological activity than the free ligands (Agwara et al., 2011; Aull et al., 1980; Ejidike and Ajibade, 2015; Lv et al., 2006; Villarreal et al., 2015). 1-Thiocarbamoylpyrazoline derivatives are well-known N,S-chelating ligands of metal ions and associate with the metal ion through the sulfur atom and the pyridine-like nitrogen (N_2) of the pyrazolyl moiety, yielding a stable five-membered ring (Budakoti et al., 2007; Evans et al., 2004; Husain et al., 2008). For this reason, this class of ligands may be particularly suitable for preparation of complexes with promising biological activities. *In vivo* antiamoebic activity against *Entameoba histolytica* A was tested with a variety of 1-thiocarbamoyl-2-pyrazoline derivatives and their palladium(II) complexes with the palladium complexes showing stronger antiamoebic properties than the non-complexed derivatives (Abid and Azam, 2005a, 2005b). 1-Thiocarbamoylpyrazolyl chelates of palladium(II) have been shown to display antitumor properties (Casas et al., 2008). Complexes of the type $[PdX_2(tdmPz)]$ {X = Cl, Br, I or SCN; tdmPz = 1-thiocarbamoyl-3,5-dimethylpyrazole}) were tested for cytotoxicity against mammary adenocarcinoma (LM3 and LMM3) and lung adenocarcinoma (LP07). The cytotoxic effect against LM3 was comparable to that of cisplatin (Rocha et al., 2010). Furthermore, certain pyrazoline coordination compounds with various metal ions possess antimicrobial properties (Al-Jibori and Anaammajeedrasheed, 2013) greater than the activity of the free ligand (Elgazwy et al., 2012). Coordination compounds of thiocarbamoyl dihydropyrazoles with nickel and copper salts have shown good inhibitory activity against several strains of the genus *Candida* with decreasing activity such that CuL > NiL > L (Ali et al., 2012). 3,5-Diaryl substituted 2-thiocarbamoyl pyrazolines form stable coordination compounds with Cu(II), Ni(II) and Fe(III), which are able to bind to DNA (Saleem et al., 2013) and showed moderate antiproliferative activity against MCF-7 cell lines. Thiocarbamoyl pyrazoline derivatives containing a pyridine moiety in the 3-position are capable of forming complexes with gold, some of which are more cytotoxic than cisplatin against the HeLa cell line (Wang et al., 2011).

In the present work, we describe a series of 3-(pyridine-2-yl)-4,5-dihydro-1H-pyrazole-1-thiocarboxamides and their copper(II) complexes with potent biological activity.

2. Experimental part

2.1. Materials and methods

All preparations were carried out in the reagent grade solvents. All chemicals used in the syntheses were obtained from Acros or Aldrich and were used without further purification. The progress of all reactions was monitored on Silufol precoated silica gel plates (with fluorescence indicator UV254) using ethyl acetate/*n*-hexane as solvent system. Melting points (mp) were taken in open capillaries on a Stuart melting point apparatus SMP11 and are uncorrected. Elemental analyses were made on a Vario MICRO cube CHNS/O Elementar. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer using $CDCl_3$ as a solvent.

X-ray diffraction was measured with Bruker APEX II CCD diffractometer at 120(2) K (graphite monochromator, (MoKα) = 0.71073 Å, -scan type) and STOE STADIVARY Pilatus-100K diffractometer at 295 K (focusing mirrors, (CuKα) = 1.54186 K diffractometer at Å, /-scan type).

Electrochemical studies were carried out on a PI-50-1.1 potentiostat in DMF. 0.05 M Bu_4NClO_4 solution in DMF served as supporting electrolyte; Ag/AgCl/KCl (satur.) was used as a referenced electrode. All measurements were carried out under argon. The samples were dissolved in pre-deaerated solvent.

Chalcones **1a–e** were synthesized according to the described methods: (Prasad et al., 2008) for **1a**, (Marvel et al., 1955) for **1b**, (Albaladejo and Alonso, 2013) for **1c**, (Coates et al., 1996) for **1d**, (Gilman and Cason, 1950) for **1e**,**f**.

5-(3,4-Dimethoxiphenyl)-3-(pyridin-2-yl)-1-thiocarbamoyl-4,5-dihydro-1*H*-pyrazole (**3a**). A mixture of 1 eq. of chalcone **1a** and 2 eq. of thiosemicarbazide in ethanol was heated until complete dissolution, then 10% aqueous solution of KOH (2 eq.) was added dropwise and the resulting solution was refluxed for 6–12 h until the reaction was completed (control by TLC). The reaction mixture was cooled, partly evaporated. The resulting precipitate was filtered and recrystallized from ethanol giving a beige powder, yield 40%, mp 182 °C. ^{1}H NMR ($CDCl_3$): 3.49 (1H, m, H^4), 3.86 (3H, s, CH_3O), 3.88 (3H, s, CH_3O), 3.94 (1H, dd, J = 18.7, 11.5 Hz, H^4), 6.04 (1H, dd, J = 11.6, 3.7 Hz, H^5), 6.15 (1H, bs, NH_2), 6.80 (3H, m, H^{Ar}), 7.15 (1H, bs. NH_2), 7.36 (1H, m, H^{Py}), 7.79 (1H, t, J = 7.8 Hz, H^{Py}), 8.07 (1H, d, J = 8.00 Hz, H^{Py}), 8.65 (1H, d, J = 4.7 Hz, H^{Py}). Calculated for $C_{17}H_{18}N_4O_2S$: C 59.63%, H 5.30%, N 16.36%, S 9.36%; found: C 59.31%, H 5.53%, N 16.15%, S 9.36%.

2.1.1. Synthesis of 1-(N-phenylthiocarbamide) 2-pyrazolines

2.1.1.1. General procedure.

1. *3-Pyridyl-pyrazolines **2b–f***. To a hot solution of 2 eq. of hydrazine hydrate in ethanol 1 eq. of chalcone **1b–f** was added in several portions with stirring; then 2 eq. of acetic acid was added dropwise. The resulting mixture was refluxed for 2–5 h to complete the reaction (TLC control), cooled, partially evaporated and poured onto ice. The precipitate was washed with water and used immediately in subsequent reaction.
2. *1-(N-phenylthiocarbamide) 2-pyrazolines **3b–f***. Freshly prepared pyrazoline **2b–f** (1 eq.) was dissolved in benzene and phenyl isothiocyanate (1.5 eq.) was added. The resulting mixture was stirred with a magnetic stirrer until the reaction was completed (TLC control). The reaction mixture was concentrated in vacuo, and the residue was dissolved in a minimum amount of ethanol, cooled, triturated with ether to form a precipitate. The precipitate was washed with ether and recrystallized from ethanol.

5-(3,4-Dimethoxiphenyl)-3-(pyridin-2-yl)-1-phenylthiocarbamoyl-4,5-dihydro-1*H*-pyrazole (3b). The yellowish-white powder, yield 41%, mp 134 °C. ^{1}H NMR ($CDCl_3$): 3.48 (1H, dd, J = 18.6, 3.6 Hz, H^4), 3.83 (3H, s, CH_3O), 3.85 (3H, s, CH_3O), 3.96 (1H, dd, J = 18.6, 11.9 Hz, H^4), 6.18 (1H, dd, J = 11.6, 3.7 Hz, H^5), 6.80 (3H, m, H^{Ar}), 7.22 (1H, m, H^{Ar}), 7.39 (3H, t, H^{Ar}, J = 7.6 Hz, H^{Py}), 7.67 (2H, d, J = 8.1 Hz, H^{Ar}), 7.82 (1H, t, J = 7.6 Hz, H^{Py}), 8.1 (1H, d, J = 7.8 Hz, H^{Py}), 8.67 (1H, d, J = 4.6 Hz, H^{Py}), 9.31 (1H, s, NH). Calculated for $C_{23}H_{22}N_4O_2S$: C 66.01%, H 5.30%, N 13.39%, S 7.66%; found: C 66.52%, H 5.84%, N 13.03%, S 7.33%.

5-Phenyl-3-(pyridin-2-yl)-1-phenylthiocarbamoyl-4,5-dihydro-1*H*-pyrazole (3c). The yellowish-white powder, yield 12%, mp 165 °C. ^{1}H NMR ($CDCl_3$): 3.48 (1H, dd, J = 18.8, 3.7 Hz, H^4), 3.99 (1H, dd, J = 18.6, 11.7 Hz, H^4), 6.24 (1H, dd, J = 11.6, 3.8 Hz, H^5), 7.22 (2H, m, H^{Ar}), 7.30 (2H, m, H^{Ar}), 7.37 (5H, m, H^{Ar} H^{Py}), 7.67 (2H, d, J = 8.0 Hz, H^{Ar}), 7.81 (1H, td, J = 7.8, 1.7 Hz, H^{Py}), 8.13 (1H, d, J = 7.8 Hz, H^{Py}), 8.66 (1H, d, J = 4.9 Hz, H^{Py}), 9.30 (1H, s, NH). Calculated for $C_{21}H_{18}N_4S$: C 70.36%, H 5.06%, N 15.63%, S 8.95%; found: C 69.90%, H 5.24%, N 15.16%, S 8.30%.

5-(4-Bromophenyl)-3-(pyridin-2-yl)-1-phenylthiocarbamoyl-4,5-dihydro-1*H*-pyrazole (3d). The white powder, yield 14%, mp 203 °C. ^{1}H NMR ($CDCl_3$): 3.41 (1H, dd, J = 18.7, 3.8 Hz, H^4), 3.96 (1H, dd, J = 18.8, 11.9 Hz, H^4), 6.14 (1H, dd, J = 11.9, 3.9 Hz, H^4), 7.18 (3H, m, $H^{Ar)}$, 7.36 (3H, br, H^{Ar}, H^{Py}), 7.44 (2H, d, J = 8.3 Hz, H^{Ar}), 7.62 (2H, d, J = 8.1 Hz, H^{Ar}), 7.78 (1H, t, J = 7.1 Hz, H^{Py}), 8.09 (1H, d, J = 7.9 Hz, H_{Py}), 8.63 (1H, d, J = 4.9 Hz, H^{Py}), 9.23 (1H, s, NH). Calculated for $C_{21}H_{17}BrN_4S$: C 57.67%, H 3.92%, N 12.81%, S 7.33%; found: C 57.74%, H 4.24%, N 12.54%, S 7.28%.

5-(4-Methylphenyl)-3-(pyridin-2-yl)-1-phenylthiocarbamoyl-4,5-dihydro-1*H*-pyrazole (3e). Yellow needle crystals, yield 46%, mp 152 °C. ^{1}H NMR ($CDCl_3$): 2.32(3H, s, CH_3), 3.49 (1H, m, H^4), 3.96 (1H, dd, J = 18.7, 11.7 Hz, H^4), 6.19 (1H, dd, J = 11.6, 3.8 Hz, H^5), 7.18 (5H, m, H^{Ar}), 7.36 (3H, m, H^{Ar}, H^{Py}), 7.65 (2H, d J = 7.6 Hz, H^{Ar}), 7.79 (1H, td, J = 7.8, 1.7 Hz, H^{Py}), 8.1 (1H, d, J = 7.9 Hz, H^{Py}), 8.64 (1H, d, J = 4.8 Hz, H_{Py}), 9.28 (1H, s, NH). Calculated for $C_{22}H_{20}N_4S$: C 70.94%, H 5.41%, N 15.04%, S 8.61%; found: C 71.30%, H 5.50%, N 14.70%, S 8.45%.

5-(3-Nitrophenyl)-3-(pyridin-2-yl)-1-phenylthiocarbamoyl-4,5-dihydro-1*H*-pyrazole (3f). Lemon-yellow crystalline powder, yield 33%, mp 155 °C. ^{1}H NMR ($CDCl_3$): 3.45 (1H, dd, J = 18.9, 4.3 Hz, H^4), 4.04 (1H, dd, J = 18.9, 12.0 Hz, H^4), 6.26 (1H, dd, J = 12.0, 4.3 Hz, H5^4), 7.21 (1H, m, H^{Ar}), 7.37 (3H, m, $H^{Ar,}$ H^{Py}), 7.52 (1H, m, H^{Ar}), 7.61 (3H, m, H^{Ar}), 7.81 (1H, td, J = 7.8, 1.7 Hz, H^{Py}), 8.12 (3H, m, H^{Ar}, H^{Py}), 8.63 (1H, d, J = 4.3 Hz, H^{Py}), 9.26 (1H, s, NH). Calculated for $C_{21}H_{17}N_5O_2S$: C 62.52%, H 4.25%, N 17.36%, S 7.95%; found: C 62.51%, H 4.50%, N 17.05%, S 8.20%.

2.1.2. Synthesis of coordination compounds

2.1.2.1. General procedure. To a solution of ligand **3** (1 mmol) in 1–2 mL of methylene chloride 0.5–1 mL of methanol was added to form a two-phase system. Then the solution of $CuCl_2{\cdot}2H_2O$ (1 mmol) in 1–2 mL of methanol was slowly poured. The reaction mixture was sealed and left to precipitation. The precipitate was filtered off, washed with cold methylene chloride, then with cold methanol/water mixture and dried under vacuum with heating. If no precipitate formed, the complex was reprecipitated by diethyl ether vapor diffusion in a closed vessel.

{[5-(3,4-Dimethoxiphenyl)-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]-carbothioamide}copper(II) dichloride (4a). Dark green crystals yield 44%, mp 180 °C (dec.). Calculated for $C_{17}H_{18}Cl_2CuN_4O_2S{\cdot}H_2O$: C 41.26%, H 4.07%, N 11.32%, S 6.48%; found: C 41.04%, H 3.86%, N 11.28%, S 6.89%.

[5-(3,4-Dimethoxyphenyl)-3-(pyridin-2-yl)-1-phenylthiocarbamoyl-4,5-dihydro-1*H*-pyrazole]copper(II) dichloride (4b). Brown powder, yield 76%, mp 200 °C (dec.). Calculated for $C_{23}H_{22}Cl_2CuN_4O_2S$: C 49.96%, H 4.01%, N 10.13%, S 5.80%; found: C 49.84%, H 4.19%, N 9.82%, S 6.02%.

[5-Phenyl-3-(pyridin-2-yl)-1-phenylthiocarbamoyl-4,5-dihydro-1*H*-pyrazole]copper(II) dichloride (4c). Dark green crystals yield 56%, mp 190–195 °C (dec). Calculated for $C_{21}H_{18}Cl_2CuN_4S$: C 51.17%, H 3.68%, N 11.37%, S 6.50%; found: C 50.94%, H 3.91%, N 11.06%, S 6.22%.

[5-(4-Bromophenyl)-3-(pyridin-2-yl)-1-phenylthiocarbamoyl-4,5-dihydro-1*H*-pyrazole]copper(II) dichloride (4d). Light green powder, yield 33%, mp 198–199 °C (dec). Calculated for $C_{21}H_{17}BrCl_2CuN_4S$: C 44.11%, H 3.00%, N 9.80%, S 5.61%; found: C 43.95%, H 3.28%, N 9.50%, S 5.35%.

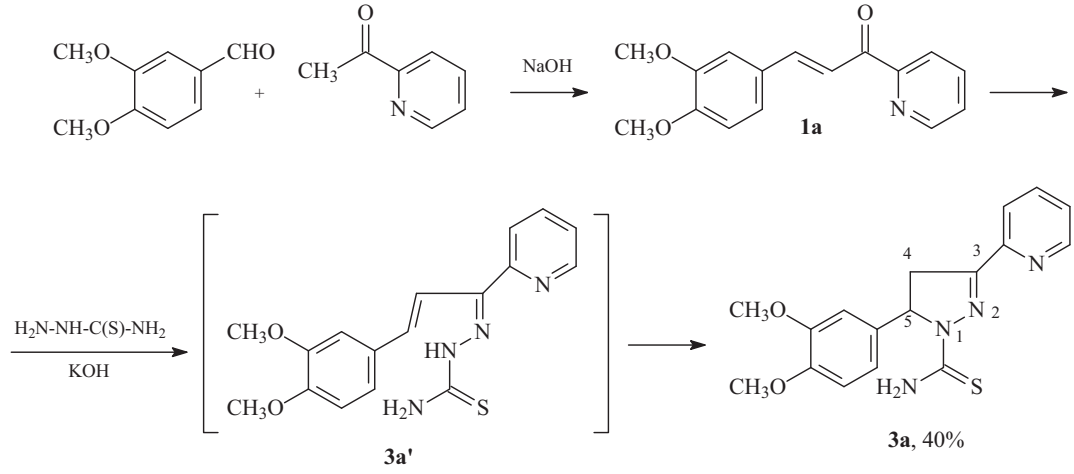
[5-(4-Methylphenyl)-3-(pyridin-2-yl)-1-phenylthiocarbamoyl-4,5-dihydro-1*H*-pyrazole]copper(II) dichloride (4e). Light green powder, yield 39%, mp 180–182 °C (dec.). Calculated for $C_{22}H_{20}Cl_2CuN_4S$: C 52.12%, H 3.98%, N 11.05%, S 6.33%; found: C 51.95%, H 4.25%, N 10.81%, S 5.99%.

[5-(3-Nitrophenyl)-3-(pyridin-2-yl)-1-phenylthiocarbamoyl-4,5-dihydro-1*H*-pyrazole]copper(II) dichloride (4f). Dark green crystals, yield 85%, mp 195–200 °C (dec). Calculated for $C_{21}H_{17}Cl_2CuN_5O_2S$: C 46.89%, H 3.19%, N 13.02%, S 5.96%; found: C 46.75%, H 3.14%, N 12.90%, S 6.13%.

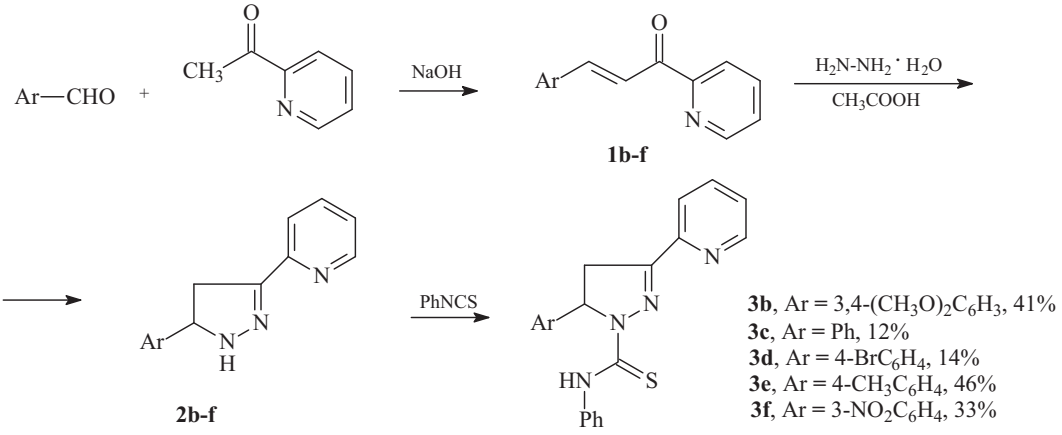
3. Results and discussion

3.1. Synthesis of ligands and complexes

The synthesis of 1-thiocarbamoyl pyrazoline derivatives **3a–f** was performed with 2-acetyl pyridine chalcones **1a–f** (Schemes 1, 2) as starting materials. The 1,3-diaryl-2-propen-1-ones chalcones **1a–f** were synthesized according to previously described methods (Albaladejo and Alonso, 2013; Coates et al., 1996; Gilman and Cason, 1950; Marvel et al., 1955; Prasad et al., 2008). Condensation of 2-acetyl pyridine-based chalcone **1a** with thiosemicarbazide in the presence of KOH yielded 5-(3,4-dimethoxyphenyl)-3-(pyridin-2-yl)-4,5-dihydro-1*H*-pyrazol-1-carbothioamide **2** (Scheme 1). The chalcones **1b-f** reacted with hydrazine hydrate giving 3-pyridyl-pyrazoline derivatives **2b–f**, which were reacted with phenyl isothiocyanate to yield the target phenylthiocarbamoyl-dihydropyrazoles **3b–f** (Scheme 1). Using this method, products **1–10** were synthesized with moderate yields. Crude products were crystallized from ethanol. The structures of the compounds were characterized by ^{1}H NMR spectroscopy. The methylene protons and the methine proton in positions 4 and 5, respectively, of the dihydro-(1*H*)-pyrazole ring, give rise to a well-defined system of three double doublets at 3.5–6.0 ppm, indicating not only the formation of the pyrazoline, but also the exact position of the C = N double bond.



Scheme 1 Synthesis of 5-(3,4-dimethoxyphenyl)-3-(pyridin-2-yl)-4,5-dihydro-1H-pyrazol-1-carbothioamide **3a**.

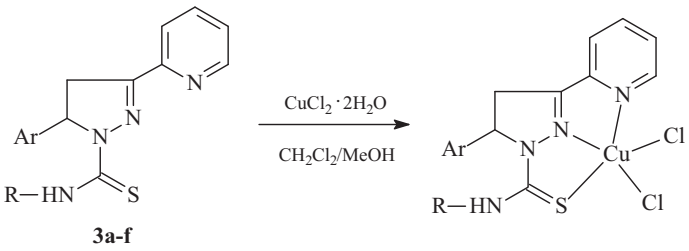


Scheme 2 Synthesis of 1-(N-phenylthiocarbamoyl)-pyrazolines **3b–f**.

Apparently, chalcone **1a** reacted with thiosemicarbazide in two stages. In the first step thiosemicarbazone **3a′** is obtained, which then cyclized forming the pyrazoline derivative **3a**. In the ^{1}H NMR spectrum of the latter compound there are the signals of two non-equivalent H4 protons of pyrazoline cycle at 3.5 and 3.9 ppm; by virtue of the fact that the H5 proton of pyrazoline cycle has a strong impact of the neighboring thioamide group, its signal is observed in the ^{1}H NMR spectrum at 6.0 ppm, which is different from the average value of the type of signals (signal shifted downfield to a ~1 ppm). Signals of NH_2 group appear as two low-intensity broadened peak at 6.15 and 7.10 ppm. In the IR spectrum of **3a** NH_2-group provides characteristic absorption band at ~3500 cm^{-1}.

A series of Cu(II) complexes with the obtained phenylthio carbamoyl-pyrazolines **3a–f** was synthesized by direct reaction of ligands, dissolved in CH_2Cl_2, with copper(II) chloride dissolved in CH_3OH (Scheme 3). All complexes were characterized by elemental analysis data. The crystal structures of complexes **4a** and **4c** were confirmed by X-ray diffraction. Coordination compounds with the composition $LCuCl_2$ (with L = 3-(pyridine-2-yl)-4,5-dihydro-1H-pyrazole-1-thiocarboxamide) were isolated for all ligands. The copper atom formed two five-membered chelate rings because of coordination between the nitrogen atoms of the pyrazoline and pyridine moieties and the sulfur atom of the thiocarbamoyl substituent. This is similar to structures previously observed for other copper complexes with the ligands of this structure (Abid and Azam, 2005a, 2005b; Budakoti et al., 2007; Casas et al., 2008; Evans et al., 2004; Husain et al., 2008; Rocha et al., 2010; Wang et al., 2011).

The structures of complexes **4a** and **4c** were confirmed by X-ray diffraction. The crystal structures are shown in Fig. 1; selected bond lengths and angles are given in Tables 1 and 2. Copper atoms in both complexes are coordinated by the two nitrogen atoms of two five-membered rings of the organic ligand, the thiocarbonyl sulfur atom and two chlorine ions. The metal atoms form a very distorted square piramidal shape; the Cu-N distances are 2.121–2.141 Å (Cu-N_{Pyrid}) and 1.915–1.930 Å (Cu-N_{Pyraz}). The pyrazoline rings of the ligands are almost flat. The phenyl substituent at the 3rd position of the ligand moieties in complexes **4a** and **4c** is almost perpendicular to the pyrazoline plane.



4a, Ar = 3,4-$(CH_3O)_2C_6H_3$, R = H, 44%
4b, Ar = 3,4-$(CH_3O)_2C_6H_3$, R = Ph, 76%
4c, Ar = R = Ph, 56%
4d, Ar = 4-BrC_6H_4, R = Ph, 33%
4e, Ar = 4-$CH_3C_6H_4$, R = Ph, 39%
4f, Ar = 3-$NO_2C_6H_4$, R = Ph, 85%

Scheme 3 Synthesis of copper(II) complexes of 1-thiocarbamoyl-pyrazolines **4a**–**f**.

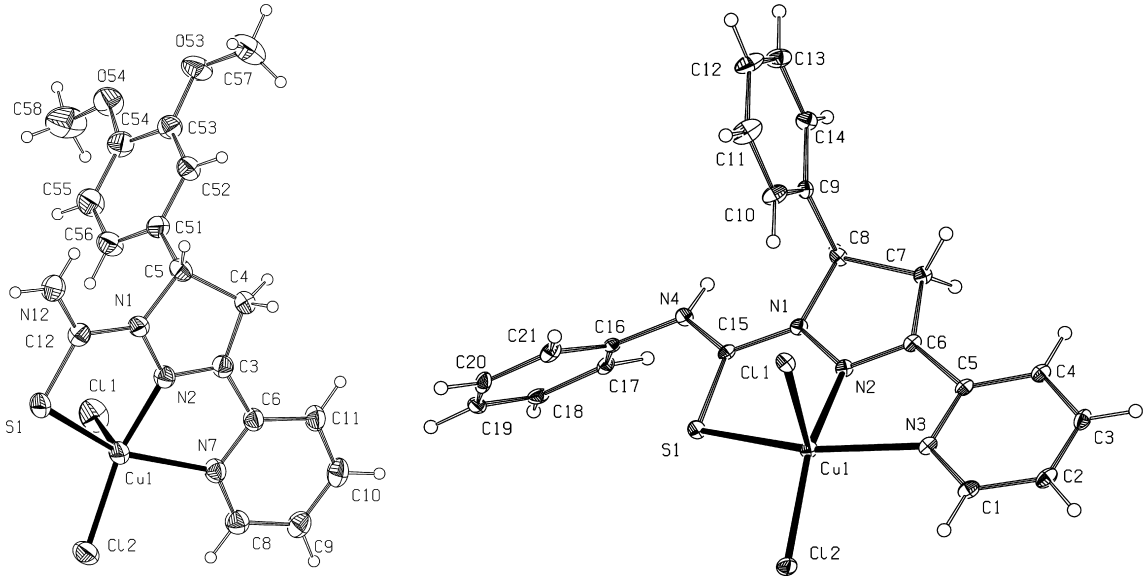


Fig. 1 Molecular structures of coordination compounds **4a** *(left)* and **4c** *(right)*.

Table 1 Selected bond lengths for coordination compounds **4a, 4b**.

Compound	Cu-Cl1	Cu-Cl2	Cu-N(Pyrid)	Cu-N(Pyraz)	Cu-S
4a	2.4730(15)	2.2164(14)	2.141(3)	1.915(4)	2.3628(13)
4c	2.5071(8)	2.2032(8)	2.121(2)	1.930(2)	2.3508(8)

3.2. Electrochemistry

Electrochemical studies were carried out for the ligands **3a–f** and their copper complexes **4a–f** using cyclic voltammetry (CV) and rotating disk electrode (RDE) techniques, with glassy carbon and gold electrodes in DMF in the presence of 0.1 M Bu_4NClO_4 as supporting electrolyte. The potentials of the electrochemical oxidation and reduction, measured relative to Ag|AgCl|KCl (sat.) are presented in Table. 3. The cyclic voltammograms are shown in Figs. 2, 3 and in Supplementary Information.

All ligands, except **3f**, were reduced by the glass carbon electrode in two irreversible stages, during the first of which one electron was gained, and the second of which two electrons were gained. Ligands **3b** and **3e**, containing electron-donating substituents in the phenyl ring, as well as unsubstituted ligand **3c**, are reduced at very similar potential values (see Table 3 and Fig. 2). Electron-withdrawing substituents have a greater influence on the potentials of cathodic peaks than other substituents. Compound **3d,** with a *p*-bromophenyl substituent, was reduced with 90 mV less than **3c**, which is due to the

Table 2 Selected angles for coordination compounds **4a, 4b**.

Compound	Cl1-Cu-Cl2	Cl1-Cu-N (Pyrid)	Cl1-Cu-N (Pyraz)	Cl2-Cu-N (Pyrid)	Cl2-Cu-N (Pyraz)	N-Cu-N	N(Pyrid)-Cu-S	N(Pyraz)-Cu-S	Cl-Cu-S
4a	106.20(6)	89.57(11)	95.62(11)	98.24(11)	157.50(12)	76.11(15)	154.11(12)	80.91(11)	98.46(5) 104.53(6)
4c	111.25(3)	90.14(7)	98.97(7)	91.47(7)	156.92(8)	76.16(10)	155.61(7)	81.55(7)	97.67(3) 97.61(10)

Table 3 Electrochemical reduction (E_{Red}) and oxidation (E_{Ox}) potentials of ligands and complexes, measured relatively to Ag|AgCl| KCl (sat.) by cyclic voltammetry (E_p is the peak potential) at glassy carbon and Au electrodes. DMF, 0.1 M Bu_4NClO_4, 200 mV/s. The values after the slash marks represent the peak potentials for the reverse CV scans.

Compound	Electrode	E_pRed, V	E_pOx, V
3a	GC	−1.80	1.18
		−2.34	1.57
	Au	−1.80	1.34
			1.62
4a	GC	0.44/0.55	1.18
		0.32/0.55	1.29
		−0.29	
		−1.79	
		−2.01	
	Au	0.35/0.44	0.87
		0.02	1.56
		−0.70	
		−1.99	
		−2.02	
		−2.22	
3b	GC	−1.67	1.23
		−2.20	1.54
	Au	−1.66	1.40
			1.57
4b	GC	0.28/0.52	0.97
		−0.82	1.33
		−1.99	
		−2.21	
		−2.38	
	Au	0.39/0.68	1.07
		−1.01	1.31
		−1.96	
3c	GC	−1.64	1.27
		−2.14	1.37
	Au	−1.67	1.46
4c	GC	0.39/0.52	1.18
		−1.69	1.31
		−1.96	
	Au	0.33/0.42	1.38
		−0.16	
		−0.69	
		−1.70	
3d	GC	−1.55	1.40
		−2.08/1.13	
	Au	−1.65	1.57
		−1.88	
		−2.19	

(*continued on next page*)

Table 3 (*continued*)

Compound	Electrode	E_pRed, V	E_pOx, V
4d	GC	0.42/0.55	1.08
		−0.70	1.21
		−1.63	1.39
		−1.93	1.60
		−2.20	
	Au	0.31/0.42	0.77
		−0.63	1.14
		−1.64	1.43
		−1.93	1.55
3e	GC	−1.67	1.28
		−2.21	1.38
	Au	−1.67	1.49
		−2.21	
4e	GC	0.41/0.53	1.05
		−1.67	1.32
		−1.98	1.52
		−2.25	
	Au	0.30/038	1.02
		−0.61	1.36
		−1.69	1.46
		−1.97	
3f	GC	−1.07/−1.00	1.45
		−2.03	
		−2.26	
	Au	−1.07/−1.01	1.60
		−1.62	
		−2.25	
4f	GC	0.44/0.54	1.08
		−0.75	1.16
		−1.11/−1.03	1.36
		−1.93	
		−2.02	
		−2.22	
	Au	0.25/0.49	0.77
		−0.21	1.13
		−0.60	1.45
		−1.12/−1.03	
		−2.06	

acceptor effect of the bromine atom in the phenyl ring. A reoxidation peak of Br^- at 1.13 V was observed for compound **3d** after the second reduction step during the reverse scan (Fig. 2d), indicating reductive cleavage of the bromine anion. Compound **3f**, with the nitro substituent on the phenyl ring, was reduced in three stages and at a lower cathodic potential value (570 mV) than the unsubstituted thiocarbamoylpyrazoline **3c**. Unlike other ligands, the first stage of reduction of compound **3f** was reversible, which, according to the reduction potential value, occurs on the non-conjugated nitro group (Butin et al., 1992).

Oxidation potentials of ligands **3a–f** are more dependent on the nature of the substituents rather than the reduction potentials. For ligands **3b** and **3e**, with donor substituents at the phenyl ring, a cathode shift of oxidation potential was observed. For **3d** and **3f**, anode shifts were observed for compounds with electron accepting substituents (Table 3).

The CV curves of coordination compounds **4a–f** from the GC electrode show the first reversible or quasi-reversible reduction peaks in the anode region at 0.45–0.28 V, corresponding to Cu(II) → Cu(I) transitions; subsequent irreversible low-intensity peaks correspond to Cu(I) → Cu(0) transitions. Cu(I) and Cu(0) containing intermediates are stable at these conditions.

In contrast, only the complexes **4a**, **4b** and **4e**, with donor substituents at the aromatic ring, were stable in the anodic and cathodic regions during reduction with the Au electrode (Fig. 3, *left*). Complexes **4d** and **f**, containing bromine or nitro groups, were decomposed by oxidation followed by release of copper metal, as evidenced by the desorption peaks of copper metal from the electrode surface on the CV curves during the reverse potential scan at 1.8 V (Fig. 3, *center*). Complexes were most likely decomposed after the oxidation, resulting in a ligand moiety oxidation product and $CuCl_2$. For the latter, the reverse scan CV curve increase is due to the reduction of free $CuCl_2$ to CuCl, which is unstable with metal electrodes and is disproportionate in terms of free metal release ($CuCl \rightarrow CuCl_2 + Cu$) (Beloglazkina et al., 2006, 2008, 2009; Majouga et al., 2009).

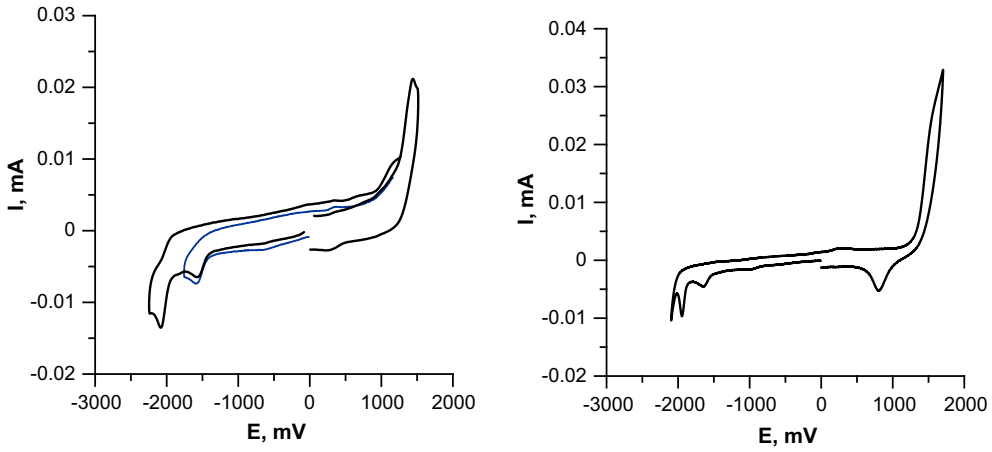


Fig. 2 CV for ligand **3e** on GC (*left*) and Au (*right*) electrodes (10^{-3} M, DMF, Bu_4NClO_4).

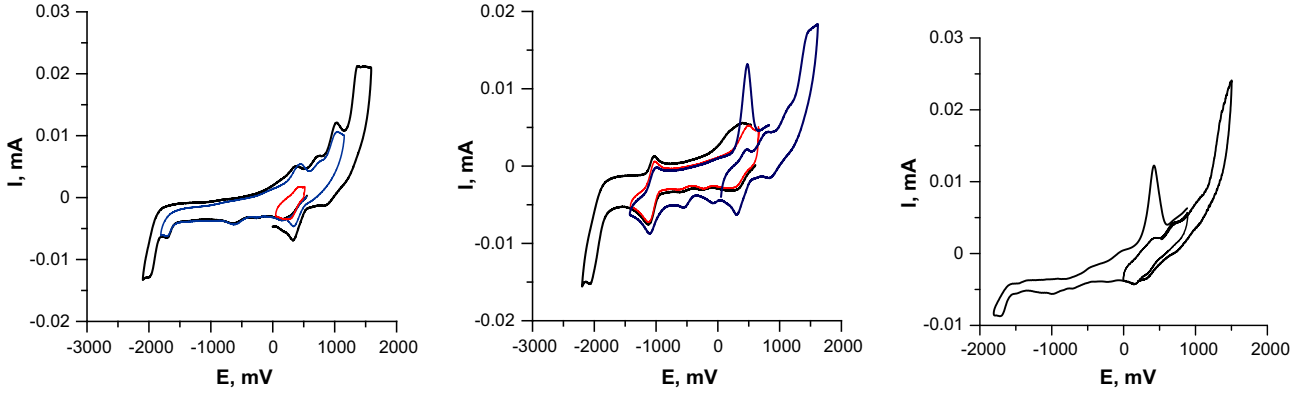


Fig. 3 CV for complexes **4e** (*left*), **4f** (*center*) and **4c** (*right*) on Au electrodes (10^{-3} M, DMF, Bu_4NClO_4).

Table 4 Cytotoxicity of ligands and complexes against A549, MCF7, HEK293T and VA13 cell lines measured by MTT-assay (μM).[a]

Compound	A549, μM	MCF7, μM	HEK293T, μM	VA13, μM
4a	28.8 ± 5.5	48.4 ± 43	19.1 ± 3.3	39.6 ± 5.5
4c	11.6 ± 1.7	9.9 ± 0.3	8.0 ± 1.5	11.0 ± 2.1
4d	14.4 ± 1.5	24.1 ± 1.7	7.4 ± 2.5	9.4 ± 0.3
4e	9.5 ± 0.8	12.1 ± 1.5	8.1 ± 1.3	8.2 ± 0.5
4f	20.7 ± 0.8	23.2 ± 0.9	12.9 ± 2.2	19.2 ± 3.7
Doxorubicin[b]	2.0 ± 0.8	2.1 ± 0.8	1.1 ± 0.1	
Cisplatin[b]	> 30	64.13 ± 3.9	12.4 ± 3.9	
$CuCl_2{\cdot}2H_2O$[c]		> 100		

[a] $CuCl_2{\cdot}2H_2O$ and $CoCl_2{\cdot}6H_2O$ have IC_{50} more then 100 μM on the cell line Hek293T (the most sensitive cell line in our tests).
[b] Majouga et al. (2014).
[c] Zhong et al. (2015).

For complex **4c**, with an unsubstituted phenyl ring in the ligand fragment, a Cu(0) desorption peak was observed after the reduction in the complex during the CV reverse scan at the −1.8 V potential (Fig. 3, *right*). We found that Cu(0)-containing reduction products are unstable in this case and degrade by forming free ligand, metallic copper and chloride anions ($[Cu^0LCl_2]^{2-} \rightarrow Cu^0 + L + 2Cl^-$).

Thus, coordination compounds **4a–f** are oxidized "to ligand" and reduced "to metal" in two stages, the first of which is reversible or quasi-reversible and the second of which is irreversible and leads to the formation of $[Cu^0LCl_2]^{2-}$. The further behavior of the reduction intermediate depends on the nature of the substituents in the ligand fragments and on the electrode material.

3.3. Cytotoxicity

Synthesized copper complexes were tested for their *in vitro* cytotoxicity against human lung cancer (A549), breast adenocarcinoma (MCF-7), human embryonic kidney (HEK293) and SV40-transformed human embryonic lung fibroblast (VA13) cells using a standard MTT assay (Mosmann, 1983). The comparison of the obtained result with the data for copper(II) chloride and clinically used drugs cisplatin and doxorubicin for copper complexes **4a–f** is shown in Table 4.

Coordination of metal ions was required for cytotoxicity of the tested compounds. The measured IC_{50} values for free ligands were much higher (> 200 μM) than the values for corresponding copper complexes. The cytotoxicity of the copper complexes with thiocarboxamide ligands was also substantially higher than the cytotoxicity of copper chloride. The nature of substituent at the thioamide nitrogen atom is important for cytotoxicity: complex **4a**, with an unsubstituted amide, was 2–4 times less cytotoxic than complexes **4c–f**, with an N-aryl-substituted ligand. Compound **4f**, containing a 3-nitrophenyl substituent at the thioamide nitrogen atom, was also less active than other N-arylthioamides (**4c**–**e**). HEK293 was most affected by the tested compounds. The cytotoxicity of complexes **4c–e** toward all tested cell lines was higher than that of cisplatin.

4. Conclusions

As a result of this research, a series of novel tridentate organic ligands 1-(N-phenylthiocarbamoyl)-pyrazolines and their coordination compounds with copper chloride were synthesized. The characterization of ligands and coordination compounds was based on NMR, elemental analysis, and cyclic voltammetry data. Two copper complexes were characterized by single-crystal X-ray analysis. The cytotoxicity of copper containing complexes, comparable to cisplatin, was demonstrated for cancer cell lines MCF-7, A549, HEK293 and VA13.

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Appendix A. Supplementary material

CCDC-1470442 and CCDC-1485872 contain the supplementary crystallographic data for **4a** and **4c**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.arabjc.2017.01.005.

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